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EFFECT OF SPRAY DRYING TEMPERATURE ON THE ACTIVITY OF α-AMYLASE

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Summary. The inactivation of fungal enzyme α-amylase due to spray drying was investigated. Maltodextrin was used as a support material. Solutions containing the enzyme were dried at four different inlet air temperatures. The activity of dried enzyme was stated as a relative activity in comparison with liquid enzyme. The Student Newman-Kelus test was made to find the significance of influence that drying temperature has on α-amylase activity. Changes of activity in relation to temperature were described by an Arrhenius-type equation.

K e y w o r d s: enzyme, activity, inactivation kinetics, spray drying.

INTRODUCTION

Enzymes are some of the most important proteins in living organisms. Most enzymatic reactions proceed – under optimal conditions – from 10^8 to10^{11} times more rapidly than the corresponding nonenzymatic reactions. Food industry has been taking advantage of enzymes as processing aids for more than fifty years [10]. α-amylase has a number of important commercial applications in the starch (starch saccharification), brewing (to increase the fermentability of beer worts), alcohol (to reduce fermentation time) and cereal food industry. However, stability and activity of enzymes are required during storage and use to promote their industrial applications [2].

Spray drying is an attractive method used for preparing active dried enzymes, which allows to maintain their quality during process. This method of dehydrating differs significantly from the others. The time of drying the droplet is very short.

Even if high inlet air temperature is applied rapid evaporation maintain a low droplet temperature, which allows spray-drying very heat sensitive products [3]. It is generally known that inactivation of enzymes during drying is typically due to thermal inactivation. But only some studies predicted the destruction of the enzyme α-amylase as a function of drying air temperature during spray-drying.

The purpose of this work was to investigate inactivation of α-amylase during spray-drying at different inlet and outlet air temperatures.

MATERIALS AND METHODS

Enzyme

Fungal α-amylase Fungamyl 800L obtained from a selected strain of Aspergillus oryzae supplied by Novozymes A/S was used. It was available as a brown liquid with a density of 1.25 g/ml. Its activity was 800 Fungal α-Amylase Units/g (FAU). One FAU is the amount of enzyme which breaks down 5.26 g starch dry matter per hour under standard conditions (temperature 37±0.5°C; pH 4.7; Ca^{2+} content 364 ppm)

Reagents and carrier material

According to research done by Meerdink and van't Riet [8], Okelo *et al.* [9] and Belghith *et al.* [1], maltodextrin (DE 6) was used as a support matrix material in this work. Due to its high concentration of dextrins it was used as a substrate in determination of enzyme activity. Other reagents applied were: $CaCl_2$, J_2, KJ, NaCl, HCl, KH_2PO_4, $Na_2HPO_4*12H_2O$ and distilled water.

Drying experiments

Experiments were performed in a laboratory spray drier (Anhydro, Denmark) at inlet air temperatures 160, 180, 200 and 220°C. Experiment for each of these temperatures was repeated 3 times. When inlet temperature achieved required value the drier was fed with distilled water until thermal equilibrium was reached. Both temperatures (inlet and outlet) were kept constant during the process. Air velocity and flow rate of the feed solution were controlled during drying and were respectively 2.3 m/s and 1.26 cm^3/s. In each experiment enzyme was dried in maltodextrin solution that consisted of: maltodextrin, distilled water, enzyme (958 mg/kg maltodextrin), $CaCl_2$ (100 ppm). Initial water content was constant and equal 3.48 kg H_2O/kg dry solids. During drying humidity of outlet air was measured.

Powder analysis

The moisture content of the powder was determined by drying for 4 hours at 105°C. Moisture content was calculated from difference in weight before and after drying.

Procedure for the determination of enzyme activity

The method was based on a maltodextrin solution degradation by α-amylase and the fact that starch gives a dark blue colour with iodine. As starch is degraded the colour fades and gradually turns into a red - brown. After a defined time of incubation at standard conditions absorbance of solution is measured.

The activity of dried enzyme in FAU units was read off the standard curve. The standard curve was obtained as follows: determination of enzyme activity was made for 5 different concentrations of liquid enzyme whose activity was known, the absorbance was measured and related to enzyme activity. The equation of the model fitted by simple regression is $y = -356\ln(x) - 410.54$. The correlation coefficient equals -0.97 which indicates strong relationship between the variables. Standard curve is shown in Fig. 1.

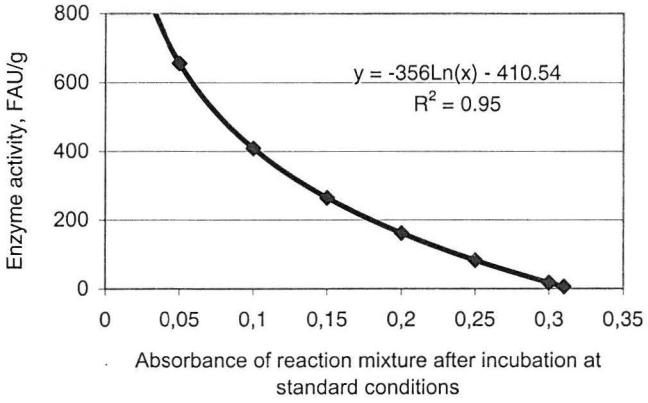

Fig. 1. Standard curve of the activity of α - amylase Fungamyl 800L.

The activity of dried enzyme was reported in FAU units and as a relative activity in comparison with liquid enzyme. The standard activity of liquid enzyme according to the product specification given by Novozymes was 800 FAU/g.

To find if there was a statistically significant difference between mean dried enzyme activities obtained at different levels of drying air temperature, analysis of variance was made. To determine which means were significantly different

from the others the multiple range test Student Newman - Kelus was made. With this method, there is a 5% risk of calling each pair of means significantly different when actual difference equals 0.

An Arrhenius - type equation was used to describe the relationship between enzyme activity and drying temperature:

$$A = A_O \, exp\left(\frac{-E_a}{RT}\right)$$

RESULTS AND DISCUSSION

Drying experiments

Outlet air temperatures obtained in triplet drying experiments performed at four inlet air temperatures are shown in Table 1.

Table 1. Outlet air temperatures for triplet experiments at four inlet air temperatures

Spray drying temperature, °C							
Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
160	64	180	65	200	71	220	90
160	62	180	64	200	73	220	90
160	58	180	68	200	77	220	88

In Table 2 some parameters obtained during experiments are shown. The average final water content in dried samples X_f ranged from 0.069 to 0.121 kg H_2O/kg dry solid. The higher drying temperature, the more water evaporated W, which resulted in decreased water content in powder. It is worth to nitice, that dry air consumption (both per second L and per kilogram of evaporated water l) was the highest for experiment performed at the lowest air temperature. Reverse dependence was observed for the influence of drying temperature on energy consumption on water evaporation (both per second Q and per kilogram of evaporated water q). These parameters significantly increased with rising of drying temperature.

Table 2. Comparison of selected drying parameters and experimental results

Parameter	Inlet air temperature, °C			
	160	180	200	220
X_i, kg H_2O/kg dry solid	3.48	3.48	3.48	3.48
X_f, kg H_2O/kg dry solid	0.121	0.122	0.095	0.069
Y_i, kg H_2O/kg dry air	0.0052	0.0052	0.0052	0.0052
Y_f, kg H_2O/kg dry air	0.0235	0.0257	0.0261	0.0262
W, kg H_2O/h	3.60	3.64	3.68	3.74
L, kg dry air/s	0.054	0.049	0.049	0.049
l, kg dry air/kg H_2O	54.4	48.8	47.8	47.5
Q, kW	4.73	4.91	5.22	6.01
q, kJ/kgH_2O	4748	4868	5108	5801

Enzyme activity

Results of α-amylase activity dried under different circumstances are given in Table 3 and presented graphically in Fig. 2. The values shown in Fig 2. are the average of triplicate experiments. In Table 3 all results and average values are given. As it is shown in the diagram and the graph drying air temperature affects dried enzyme activity. Increasing the inlet air temperature results in lower degradation of enzyme activity.

α-amylase was more sensitive to thermal inactivation during drying at lower temperatures. It resulted in higher degradation at those temperatures, which is presented in Fig. 2. The lowest sensitivity to thermal inactivation α-amylase shown during drying at inlet air temperature 220°C. The Student Newman -Kelus test showed that difference in enzyme activity between enzyme dried at inlet air temperature 160 and 220°C was statistically significant.

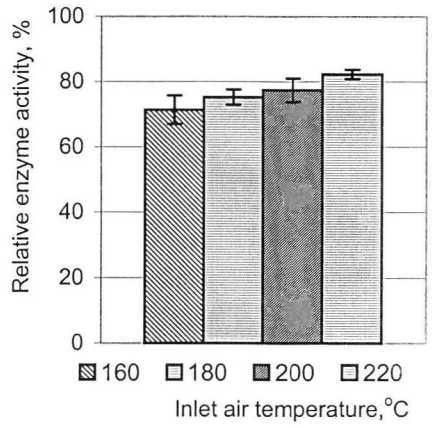

Fig. 2. Relative activity of α-amylase dried at different inlet air temperatures.

Table 3. Activity of dried α-amylase

Inlet air temperature, °C	A, FAU/g	RA, %
160	576.5± 36.3	72.1
160	533.6± 17.9	66.7
160	603.1± 8.7	75.4
160 average	571.1	71.4
180	618.8± 22.7	77.3
180	582.2± 53.4	72.8
180	606.2± 30.7	75.8
180 average	602.4	75.3
200	591.0± 16.8	73.9
200	618.8± 31.8	77.3
200	648.9± 9.5	81.1
200 average	619.6	77.4
220	666.8± 5.2	83.3
220	663.1± 10.3	82.9
220	645.4± 14.7	80.7
220 average	658.4	82.3

The results of enzyme activity dried at different inlet temperatures are shown in Fig. 3. This linear dependence confirms that the relationship between activity and drying air temperature can be described by an Arrhenius – type equation:

$$A = 3.18*10^3 \exp\left(\frac{-4.73*10^3}{RT}\right)$$

Activation energy calculated from Arrhenius equation was equal 4.73 kJ/mol. The analysis of results obtained in this work showed that the application of spray - drying allows good α-amylase activity retention. In addition this method gives possibility to further reduce enzyme degradation. It could be reached by changing inlet and outlet air temperature which were shown to have statistically significant effect on quality retention. The fact that the best activity preservation during drying was observed when drying was performed at the highest inlet and outlet temperatures can be connected with α-amylase properties as a protein substance and some operation parameters of spray-drying. It was claimed by

some authors that heat stability of α-amylase increases at lower moisture content [7,8]. Due to rapid evaporation during spray-drying water content of dried material decreases in a very short time. According to that, heat stability of α-amylase increases during the process. The higher drying air temperature the faster water evaporation, the faster α-amylase reaches its higher heat stability, and the lower inactivation is observed. In addition, rapid evaporation maintains a low droplet temperature so that high drying air temperatures can be applied without affecting the product quality. Final product's temperature is not higher than the outlet air temperature. The time of drying is very short in comparison with most of other drying processes. Low product temperature and short time allow spray drying of very heat-sensitive products [3].

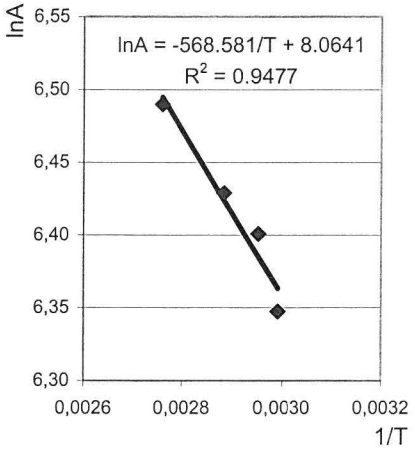

Fig. 3. Arrhenius plot for the activity of α-amylase dried at different outlet air temperatures.

Because of higher energy consumption on water evaporation in experiments when the best relative activity was reached, further experiments should be done to find optimal spray-drying conditions from the point of view of quality retention as well as suitable economic feasibility.

CONCLUSIONS

1. Spray-drying can be successfully used for dehydration of liquid α-amylase.
2. Drying air temperature affects dried enzyme activity.
3. Increasing of inlet and outlet air temperature leads to better enzyme activity retention.
4. Effect of temperature on the activity degradation during spray-drying can be expressed in the form of an Arrhenius-type equation.

NOMENCLATURE

A - enzyme activity, FAU/g
E_a - activation energy of enzyme inactivation, J/mol
L - dry air consumption, kg dry air/s

l - dry air consumption per kilogram of evaporated water, kg dry air/kg H_2O
Q - energy consumption on water evaporation, kW
q - energy consumption per kilogram of evaporated water, kJ/kg H_2O
R - gas constant, 8.318 J/mol K
RA - enzyme relative activity, %
r_e - reaction rate of enzyme inactivation
T - temperature, K
W - evaporation rate, kg H_2O/s
X_f - final water content, kg H_2O/kg dry solid
X_i - initial water content, kg H_2O/kg dry solid
Y_f - final air humidity, kg H_2O/kg dry air
Y_i - initial air humidity, kg H_2O/kg dry air

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WPŁYW TEMPERATURY SUSZENIA ROZPYŁOWEGO NA AKTYWNOŚĆ α-AMYLAZY

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Streszczenie. W pracy zaprezentowano wyniki doświadczeń mających na celu określenie wpływu temperatury wlotowej i wylotowej powietrza suszącego na stopień inaktywacji α-amylazy w czasie suszenia rozpyłowego. Roztwór enzymu i maltodekstryny, stosowanej jako materiał inertny, suszono w czterech temperaturach powietrza wlotowego: 160, 180, 200 i 220°C. Aktywność suszonego enzymu wyrażano jako aktywność względną w stosunku do aktywności enzymu płynnego. W celu określenia istotności badanej zależności między temperaturą suszenia a aktywnością enzymu wykonano analizę wariancji oraz porównania wielokrotne z zastosowaniem testu Student Newman-Kelus.

Słowa kluczowe: inaktywacja, enzym, suszenia rozpyłowe.